PB85-218873

Role of Acetylcholine in Rhythmic Spontaneous Contractions of Rat's Duodenal Smooth Muscle

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

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TECHNICAL REPORT DATA (Please read Instructions on the reverse before completing)		
1. REPORT NO. EPA/600/J-85/067	2.	3. R PB85-218873
4. TITLE AND SUBTITLE The Role of Acetylcholine in Rhythmic Spontaneous Contractions (Journal Version)		5. REPORT DATE 1985 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Ernest R. Whitcomb, BEB, EBD Andrew Stead, BB, BD		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Health Effect Research Laboratory USEPA Research Triangle Park, NC 27711		10. PROGRAM ELEMENT NO. ACSL1A 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Office of Research & Development Health Effects Research Laboratory USEPA Research Triangle Park, NC 27711		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/11
15. SUPPLEMENTARY NOTES Published In: Bulletin of Environmental Contamination and Toxicology 34(3): 476-480, March 1985		

16. ABSTRACT

The purpose of this study is reexamine the role of endogenous acetylcholine in spontaneous contractions of smooth muscle whose contractions are associated with cell metabolism. The study does not attempt to define the role of endogenous acetylcholine.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) UNCLASSIFIED	21. NO. OF PAGES 6
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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EPA/600/J-85/067
JOURNAL ARTICLE

Bull. Environ. Contam. Toxicol. (1985) 34:476–480

Environmental Contamination and Toxicology

The Role of Acetylcholine in Rhythmic Spontaneous Contractions of Rat's Duodenal Smooth Muscle

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The action of acetylcholine on smooth muscle has been the subject of numerous studies as indicated by the reviews of Paton (1964) and Schatzman (1968). Using smooth muscle from various organs these studies have suggested that exposure to acetylcholine results in initiating spontaneous activity in quiescent smooth muscle and increasing tone and amplitude of contraction in active smooth muscle. While there is agreement on some aspects of the action of acetylcholine, there is disagreement about its role as a regulator of spontaneous rhythmic contractions in small intestine. Takesi et al. (1965a,b, 1968) support a neurogenic proposal which suggests that spontaneously released acetylcholine (endogenous) from neural tissue acts as the regulatory agent. In contrast, Bozler (1948) contends that the rhythmic activity of smooth muscle is linked to cellular metabolism. This myogenic proposal is supported by Bortoff (1976) and Connor et al. (1976, 1977). However, studies of the spontaneous rhythmic contractions of smooth muscle have been limited by one or more of the following factors: (1) using smooth muscle segments which displayed a minimum of activity during the control or baseline period, (2) using short exposures and evaluating the effect visually from trace recordings, (3) requiring each smooth muscle segment to serve as its own control and, (4) lack of quantitative data for statistical analysis.

With the introduction of the intercontraction interval approach (Whitcomb et al. 1984) the above limiting factors were eliminated. In a subsequent study by Whitcomb and Stead (in preparation) the effect of temperature on duodenal smooth muscle spontaneous rhythmic contractions was evaluated in terms of an intercontraction interval mean and standard deviation for four laboratory species. It was shown that the intercontraction interval mean decreased with the same linear slope with an increase in temperature for duodenal smooth muscle segments from the rabbit, rat and mouse. The guinea pig's duodenal smooth muscle did not demonstrate a similar response. These temperature observations were interpreted as supporting a role for cellular metabolism in duodenal smooth muscle spontaneous contractions for the rabbit, rat, and mouse. Since the above study did not attempt to define the role of endogenous acetylcholine, the purpose of the present study is to reexamine the role of endogenous acetylcholine in spontaneous contractions of smooth muscle whose

contractions are associated with cell metabolism.

MATERIALS AND METHODS

The "in vitro" recording procedures and data analyses have been reported earlier (Whitcomb et al. 1984). In the present study only the mean and standard deviation of the intercontraction interval distribution were examined.

As in earlier studies only the initial segment of the duodenum was used. The mean and standard deviation in milliseconds (MS) for each gut segment was based on 512 consecutive spontaneous contractions which were measured in terms of intercontraction intervals by software using a microprocessor based system designed and constructed in this laboratory. With a mean and standard deviation for each animal, it was possible to have an average mean ± S.E. and an average standard deviation ± S.E. for a given group of animals.

Charles River, white male rats, 34 to 56 days old, from Charles River Laboratory, Kinston, Mass. were used.

The following three pharmacological agents were used: (1) physostigmine which acts indirectly by inhibiting acetylcholinesterase resulting in an increased concentration of endogenous acetylcholine, (2) exogenous acetylcholine which acts directly on smooth muscle and, (3) atropine which acts by competing with acetylcholine for cholinergic sites thus reducing the effective concentration of acetylcholine. Concentrations used are shown in the tables.

Following initial equilibration at 6 °C for one hour in modified Ringer's solution, the treated gut segments were exposed to a given compound for one hour at 37 °C prior to recording the intercontraction interval.

The variation in the estimated intercontractional interval distributional means and standard deviations among the treated groups (physostigmine, acetylcholine, and atropine) was too heterogeneous to pursue statistical analysis with traditional parametric methods, e.g. analysis of variance or regression.

Alternatively, Jonckheere's nonparametric test (Jonckheere 1954) for monotonicity was employed. This procedure tests the null hypothesis, for a given additive, that the treatment effects at each dose are equal versus the alternate hypothesis that these effects are ordered according to the concentration of the additive.

RESULTS AND DISCUSSION

The difference in age between 34, 35, and 56 days was considered to be not significant in this experimental design. Analysis of the control data supported this hypothesis.

Table 1 shows the effect of increasing concentration of physostigmine on the rhythmic contraction as reflected by the intercon-

traction interval mean and standard deviation. Using Jonckheere's test, there was a suggestion that the intercontraction interval mean decreased with increasing physostigmine concentrations ($p \cong 0.01$). An even more dramatic effect was evident for the decreasing standard deviation with increasing physostigmine concentration ($p \cong 0.001$).

Table 1. Effect of Physostigmine on the Intercontraction Interval Mean and Standard Deviation

Age (days)	Compound	Variable	Number of Animals	MS	S.E.
56	Physostigmine				
	Control	Mean	4	2072	150
		S.D.	4	704	131
	2×10^{-8}M	Mean	4	1994	72
		S.D.	4	604	86
	2×10^{-7}M	Mean	4	1947	69
		S.D.	4	336	138
	2×10^{-6}M	Mean	4	1769	30
		S.D.	4	75	7

On the basis of the physostigmine data the role of endogenous acetylcholine in the rat's duodenal smooth muscle contractions appears to be that of regulating the dispersion of contraction. While the reduction in the intercontraction interval mean suggests an increase in frequency, this may be an indirect affect. The decreased standard deviation reflected a diminished skewness to the right. As a result there are fewer intercontraction intervals larger than the mean. This results in an average intercontraction interval mean that is reduced as compared to the control. The visual impression of the trace recording is a pronounced increase in synchronization. Thus, it would appear that both the pacemaker cell's metabolism and endogenous acetylcholine are reflected in the rat's duodenal smooth muscle spontaneous contraction.

The cell's metabolism determines the slow waves (sometimes referred to as either basic electric rhythms or pacesetters potential) and the endogenous acetylcholine regulates the superimposed action potentials. Both potentials are required to initiate a contraction. Thus, the frequency reflects the cell's metabolism and the degree of synchronization reflects the level of endogenous acetylcholine.

The effect of exogenous acetylcholine on the intercontraction interval mean and standard deviation is shown in Table 2. Jonckheere's test did not indicate any trend in either the intercontraction interval mean ($p \cong 0.28$) or the standard deviation ($p \cong 0.44$) with increasing concentration.

Evaluating the exogenous acetylcholine data as to a role for endogenous acetylcholine can be misleading. Initially, the exterior surface of the smooth muscle segment is exposed before the acetylcholine can diffuse to the area of the endogenous acetylcholine.

Even after adequate equilibration the summed effects of all the sites exposed to acetylcholine may obscure the synchronization involving the endogenous acetylcholine sites.

Table 2. Effect of Acetylcholine on the Intercontraction Interval Mean and Standard Deviation

Age (days)	Compound	Variable	Number of Animals	MS	S.E.
35	Acetylcholine				
	Control	Mean	3	1837	146
		S.D.	3	640	149
	1×10^{-7}M	Mean	3	2033	237
		S.D.	3	754	247
	2×10^{-7}M	Mean	3	2267	338
		S.D.	3	767	308
	3×10^{-7}M	Mean	3	1809	23
		S.D.	3	490	26

The effect of atropine on the intercontraction interval mean and standard deviation is shown in Table 3. Again Jonckheere's test did not indicate any trend in either the intercontraction interval mean ($p \cong 0.23$) or the standard deviation ($p \cong 0.34$) with increasing concentration.

Table 3. Effect of Atropine on the Intercontraction Interval Mean and Standard Deviation

Age (days)	Compound	Variable	Number of Animals	MS	S.E.
35	Acetylcholine				
	Control	Mean	3	1842	110
		S.D.	3	621	175
	2×10^{-7}M	Mean	2	1840	146
		S.D.	2	611	185
	4×10^{-7}M	Mean	3	1736	40
		S.D.	3	515	76
	6×10^{-7}M	Mean	3	1746	85
		S.D.	3	602	74

The atropine data like the exogenous acetylcholine data did not suggest a role for endogenous acetylcholine. However, atropine's ineffectiveness in blocking the action of spontaneous released acetylcholine is consistent with the observations that large doses do not block intestinal and urinary bladder smooth muscle response to nerve stimulation. Thus, the ineffectiveness of atropine on the intercontraction interval mean and standard deviation may represent atropine's lack of access to the endogenous acetylcholine's sites.

An interpretation of these analyses has suggested that the spontan-

eous rhythmic contractions of the rat's duodenal smooth muscle is not either myogenic or neurogenic in origin rather, it is the combined action of the pacemaker cell metabolism and endogenous acetylcholine. The cell metabolism regulates the basic electric rhythm which determines when a contraction can occur. The endogenous acetylcholine initiates the superimposed action potentials on the basic electric rhythm and thus determines the regularity or synchronization of muscle contractions.

Acknowledgements. We would like to acknowledge B. Crabtree for typing the manuscript. This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Received May 7, 1984; accepted June 15, 1984.

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